

भारतीय मानक  
शुष्क दुग्ध और शुष्क दुग्ध उत्पाद — वसा का अंश ज्ञात  
करना — भारात्मक पद्धति (संदर्भ पद्धति)  
( दूसरा पुनरीक्षण )

*Indian Standard*  
DRIED MILK AND DRIED MILK PRODUCTS —  
DETERMINATION OF FAT CONTENT —  
GRAVIMETRIC METHOD (REFERENCE METHOD)  
( *Second Revision* )

ICS 67.100.10

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## NATIONAL FOREWORD

This Indian Standard (Second Revision) which is identical with ISO 1736 : 2008 'Dried milk and dried milk products — Determination of fat content — Gravimetric method (Reference method)' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Dairy Products and Equipment Sectional Committee and approval of the Food and Agriculture Division Council.

This standard was first published in 1986 under dual numbering which was identical with ISO 1736 : 1985 and revised in 2005 as ISO 1736 was technically revised in 2000. This second revision has been brought about to align the standard with the latest version of ISO 1736 : 2008, which has been technically revised.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

- a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.
- b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to certain International Standards for which Indian Standards also exist. The corresponding Indian Standards which are to be substituted in their respective places are listed below along with their degree of equivalence for the editions indicated:

<i>International Standard</i>	<i>Corresponding Indian Standard</i>	<i>Degree of Equivalence</i>
ISO 835 : 2009 Laboratory glassware — Graduated pipettes	IS/ISO 835 : 2009 Laboratory glassware — Graduated pipettes	Identical
ISO 1042 : 1998 Laboratory glassware — One-mark volumetric flasks	IS 915 : 2006 One-mark volumetric flasks	Technically Equivalent
ISO 4788 : 2005 Laboratory glassware — Graduated measuring cylinders	IS 878 : 2008 Laboratory glassware — Graduated measuring cylinders	Identical

The technical committee has reviewed the provision of the following International Standard referred in this adopted Standard and has decided that it is acceptable for use in conjunction with this standard:

<i>International Standard</i>	<i>Title</i>
ISO 3889	Milk and milk products — Specification for Mojonnier-type fat extraction flasks

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

# DRIED MILK AND DRIED MILK PRODUCTS — DETERMINATION OF FAT CONTENT — GRAVIMETRIC METHOD (REFERENCE METHOD) ( *Second Revision* )

**WARNING** — The use of this International Standard may involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish safety and health practices and determine the applicability of regulatory limitations prior to use.

## 1 Scope

This International Standard specifies the reference method for the determination of the fat content of dried milk and dried milk products. The method is also applicable to dried milk with a fat content of 40 % mass fraction or more, dried whole, dried partially skimmed, and dried skimmed milk, dried whey, dried buttermilk and dried butter serum.

The method is not applicable when the powder contains hard lumps which do not dissolve in ammonia solution or free fatty acids in significant quantities.

**NOTE** When the powder contains hard lumps which do not dissolve in ammonia solution or contains free fatty acids in significant quantities, noticeable by a distinct smell, the result of the determination will be too low. With such products, a method using the Weibull-Berntrop principle is suitable (see ISO 8262-3|IDF 124-3<sup>[3]</sup>).

## 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 835, *Laboratory glassware — Graduated pipettes*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

ISO 3889|IDF 219, *Milk and milk products — Specification of Mojonnier-type fat extraction flasks*

ISO 4788, *Laboratory glassware — Graduated measuring cylinders*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

### 3.1

#### **fat content of dried milk and dried milk products**

mass fraction of substances determined by the procedure specified in this International Standard

**NOTE** The fat content is expressed as a percentage mass fraction.

## 4 Principle

An ammoniacal ethanolic solution of a test portion is extracted with diethyl ether and light petroleum. The solvents are removed by distillation or evaporation. The mass of the substances extracted is determined.

NOTE This is usually known as the Röse-Gottlieb principle.

## 5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and only distilled or demineralized water or water of equivalent purity.

The reagents shall leave no appreciable residue when the determination is carried out by the method specified (see 9.2.2).

**5.1 Ammonia solution**, containing a mass fraction of  $\text{NH}_3$  of approximately 25 % ( $\rho_{20} = 910 \text{ g/l}$ ).

NOTE If ammonia solution of this concentration is not available, a more concentrated solution of known concentration may be used (see 9.4.2).

**5.2 Ethanol** ( $\text{C}_2\text{H}_5\text{OH}$ ), or ethanol denatured by methanol, containing a volume fraction of ethanol of at least 94 %. (See Clause A.5.)

**5.3 Congo red solution.**

Dissolve 1 g of Congo red ( $\text{C}_{32}\text{H}_{22}\text{N}_6\text{Na}_2\text{O}_6\text{S}_2$ ) in water in a 100 ml one-mark volumetric flask (6.14). Make up to the mark with water.

NOTE The use of this solution, which allows the interface between the solvent and aqueous layers to be seen more clearly, is optional (see 9.4.4). Other aqueous indicator solutions can be used provided that they do not affect the result of the determination.

**5.4 Diethyl ether** ( $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ ), free from peroxides (see Clause A.3), containing no more than 2 mg/kg of antioxidants, and complying with the requirements for the blank test (see 9.2.2, Clauses A.1 and A.4).

**WARNING — The use of diethyl ether can lead to hazardous situations. Observe current safety precautions for handling, use, and disposal.**

**5.5 Light petroleum**, with any boiling range between 30 °C and 60 °C or, as equivalent, **pentane** ( $\text{CH}_3[\text{CH}_2]_3\text{CH}_3$ ) with a boiling point of 36 °C and complying with the requirements for the blank test (see 9.2.2, Clauses A.1 and A.4).

The use of pentane is recommended because of its higher purity and consistent quality.

**5.6 Mixed solvent.**

Shortly before use, mix equal volumes of diethyl ether (5.4) and light petroleum (5.5).

## 6 Apparatus

**WARNING — Since the determination involves the use of volatile flammable solvents, all electrical apparatus employed shall comply with legislation relating to the hazards in using such solvents.**

Usual laboratory equipment and, in particular, the following.

**6.1 Analytical balance**, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

**6.2 Centrifuge**, capable of holding the fat-extraction flasks or tubes (6.6) and capable of spinning at a rotational frequency of  $500 \text{ min}^{-1}$  to  $600 \text{ min}^{-1}$  to produce a radial acceleration of  $80g$  to  $90g$  at the outer end of the flasks or tubes.

The use of the centrifuge is optional but recommended (see 9.4.7).

**6.3 Distillation or evaporation apparatus**, for distilling the solvents and ethanol from the boiling or conical flasks, or evaporating from beakers and dishes (see 9.4.14) at a temperature not exceeding  $100^\circ\text{C}$ .

**6.4 Drying oven**, electrically heated, with ventilation port(s) fully open, capable of being maintained at a temperature of  $102^\circ\text{C} \pm 2^\circ\text{C}$  throughout its working space.

The oven shall be fitted with a suitable thermometer.

**6.5 Water bath**, capable of being maintained at a temperature of  $65^\circ\text{C} \pm 5^\circ\text{C}$ .

**6.6 Mojonnier-type fat-extraction flasks**, as specified in ISO 3889|IDF 219.

NOTE It is also possible to use fat-extraction tubes with siphon or wash-bottle fittings, but then the procedure is different. The alternative procedure is given in Annex B.

The fat-extraction flasks shall be provided with good quality cork bungs or stoppers of another material (e.g. silicone rubber or polytetrafluoroethylene) unaffected by the reagents used. Cork bungs shall be extracted with the diethyl ether (5.4), kept in water at a temperature of  $60^\circ\text{C}$  or more for at least 15 min, and shall then be allowed to cool in the water so that they are saturated when used.

**6.7 Rack**, for holding the fat-extraction flasks (or tubes) (6.6).

**6.8 Wash bottle**, suitable for use with the mixed solvent (5.6).

A plastics wash bottle shall not be used.

**6.9 Fat-collecting vessels**, such as boiling flasks (flat-bottomed), of capacities 125 ml to 250 ml, conical flasks, of capacity 250 ml, or metal dishes.

If metal dishes are used, they shall be of stainless steel, flat-bottomed with a diameter of 80 mm to 100 mm and a height of approximately 50 mm.

**6.10 Boiling aids**, fat-free, of non-porous porcelain or silicon carbide (optional when metal dishes are used).

**6.11 Measuring cylinders**, of capacities 5 ml and 25 ml, complying with the requirements of ISO 4788, class A, or any other apparatus suitable for the product concerned.

**6.12 Pipettes**, graduated, of capacity 10 ml, complying with the requirements of ISO 835, class A.

**6.13 Tongs**, made of metal, for holding flasks, beakers or dishes.

**6.14 Volumetric flask**, one-mark, of capacity 100 ml, complying with the requirements of ISO 1042, class A.

## 7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707|IDF 50<sup>[1]</sup>.

Store laboratory samples at a temperature between  $2^\circ\text{C}$  and  $6^\circ\text{C}$  from the time of sampling to the time of commencing the procedure.

## 8 Preparation of test sample

Thoroughly mix the test sample by repeatedly rotating and inverting the sample container. If necessary, transfer all of the test sample to an airtight container of approximately twice the volume of the test sample to allow this operation to be carried out.

## 9 Procedure

NOTE 1 If it is required to check whether the repeatability limit (11.2) is met, carry out two single determinations in accordance with 9.1 to 9.4.

NOTE 2 An alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings (see Note to 6.6) is given in Annex B.

### 9.1 Test portion

Mix the test sample (Clause 8) by gently stirring or rotating and inverting the container several times. Immediately weigh, to the nearest 1 mg, directly or by difference, in a fat-extraction flask (6.6), one of the following test portions:

- a) about 1,000 g of dried high-fat milk, of dried whole milk or of dried butter serum;
- b) about 1,500 g of dried partially skimmed milk;
- c) about 1,500 g of dried skimmed milk;
- d) about 1,500 g of dried whey;
- e) about 1,500 g of dried buttermilk.

Transfer the test portion as completely as possible into the lower (small) bulb of the fat-extraction flask.

### 9.2 Blank tests

#### 9.2.1 Blank test for method

Carry out a blank test simultaneously with the determination using the same procedure and the same reagents, but replacing the dispersed test portion in 9.4.1 by 10 ml of water (see Clause A.2).

When one blank sample is used for a batch of test samples of which the individual samples may not have exactly the same conditions, ensure that the procedure for obtaining the value of the blank used in the calculation of the result corresponds exactly to that of the individual test sample.

If the value obtained in the blank test regularly exceeds 1,0 mg, check the reagents if this has not been recently done (9.2.2). Corrections of more than 2,5 mg should be mentioned in the test report.

#### 9.2.2 Blank test for reagents

To test the quality of the reagents, carry out a blank test as specified in 9.2.1. Additionally, use an empty fat-collecting vessel, prepared as specified in 9.3, for mass control purposes. The reagents shall leave no residue greater than 1,0 mg (see Clause A.1).

If the residue of the complete reagent blank test is greater than 1,0 mg, determine the residue of the solvents separately by distilling 100 ml of the diethyl ether (5.4) and light petroleum (5.5), respectively. Use an empty fat-collecting vessel, prepared for control purposes as in the preceding paragraph, to obtain the real mass of residue which shall not exceed 1,0 mg.

Very occasionally, the solvents may contain volatile matter which is strongly retained in fat. If there are indications of the presence of such substances, carry out blank tests on all the reagents and for each solvent using a fat-collecting vessel with about 1 g of anhydrous butterfat. If necessary, redistil solvents in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Use the solvents only shortly after the redistillation.

Replace unsatisfactory reagents and solvents, or redistil solvents.

### 9.3 Preparation of fat-collecting vessel

Dry a fat-collecting vessel (6.9) with a few boiling aids (6.10) in the oven (6.4) maintained at 102 °C for 1 h.

NOTE 1 Boiling aids are desirable to promote gentle boiling during the subsequent removal of solvents, especially when using glass fat-collecting vessels; their use is optional with metal dishes.

Protect the fat-collecting vessel from dust and allow it to cool to the temperature of the weighing room (glass fat-collecting vessel for at least 1 h; metal dish for at least 30 min).

To avoid insufficient cooling or unduly long cooling times, the fat-collecting vessel should not be placed in a desiccator.

Use tongs (6.13) to place the fat-collecting vessel on the balance. Weigh the fat-collecting vessel to the nearest 1,0 mg.

NOTE 2 The use of tongs effectively avoids, in particular, inducing temperature variations.

### 9.4 Determination

#### 9.4.1 Carry out the determination without delay.

Add about 10 ml of preheated water at a temperature of  $65\text{ °C} \pm 5\text{ °C}$  to the test portion in the fat-extraction flask (9.1) to obtain a total volume of 10 ml to 11 ml. Use the water to wash the test portion into the small bulb of the fat-extraction flask. Mix thoroughly with the test portion in the small bulb until the test portion is completely dispersed.

9.4.2 Add 2 ml of ammonia solution (5.1) to the test portion (9.4.1), or an equivalent volume of a more concentrated ammonia solution (see Note to 5.1). Mix thoroughly with the test portion in the small bulb of the fat-extraction flask.

9.4.3 Heat the flask to  $65\text{ °C} \pm 5\text{ °C}$  in the water bath (6.5) for 15 min to 20 min with occasional shaking. Cool in running water to room temperature.

9.4.4 Add 10 ml of ethanol (5.2). Mix gently but thoroughly by allowing the contents of the fat-extraction flask to flow backwards and forwards between the small and large bulb. Avoid bringing the liquid too near to the neck of the flask. If desired, add 2 drops of the Congo red solution (5.3).

9.4.5 Add 25 ml of diethyl ether (5.4). Close the fat-extraction flask with a cork bung saturated with water or with a stopper of other material wetted with water (6.6). Shake the flask vigorously, but not excessively, for 1 min to avoid the formation of persistent emulsions.

While shaking, keep the fat-extraction flask in a horizontal position with the small bulb extending upwards, periodically allowing the liquid to run from the large bulb into the small bulb. If necessary, cool the flask in running water to about room temperature. Carefully remove the bung or stopper and rinse it and the neck of the flask with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the flask.

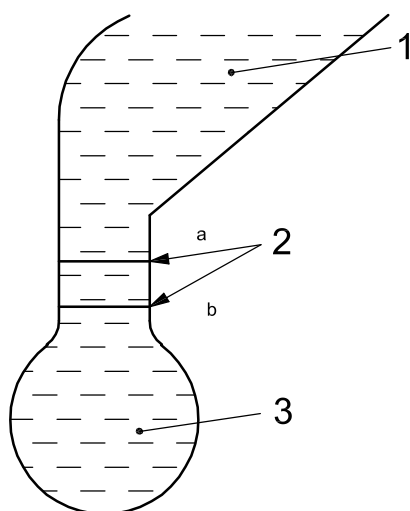
9.4.6 Add 25 ml of the light petroleum (5.5). Close the fat-extraction flask with the rewetted (by dipping into water) bung or stopper. Mix gently again for 30 s as specified in 9.4.4. Proceed with shaking as specified in 9.4.5.

**9.4.7** Centrifuge the closed fat-extraction flask for between 1 min and 5 min at a radial acceleration of 80g to 90g. If a centrifuge (6.2) is not available, allow the closed flask to stand in the rack (6.7) for at least 30 min until the supernatant layer is clear and distinctly separated from the aqueous layer. If necessary, cool the flask in running water, to room temperature.

**9.4.8** Carefully remove the bung or stopper and rinse it and the inside of the neck of the fat-extraction flask with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the flask. If the interface is below the bottom of the stem of the flask, raise it slightly above this level by gently adding water down the side of the flask (see Figure 1) to facilitate the decanting of solvent.

**NOTE** In Figures 1 and 2, one of the three types of fat-extraction flasks as specified in ISO 3889|IDF 219 has been chosen, but this does not imply any preference over other types.

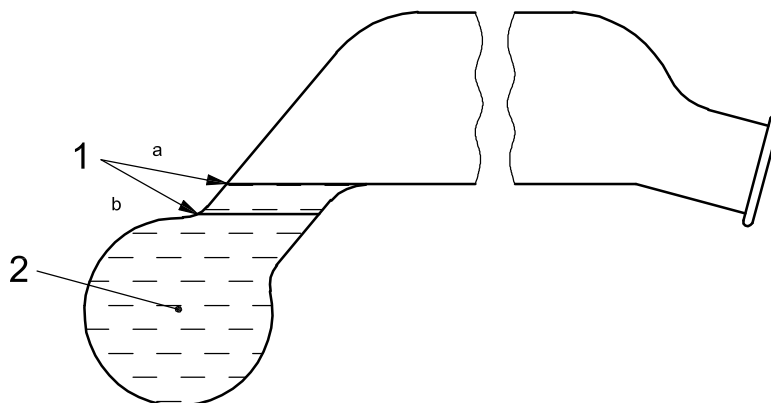
**9.4.9** Hold the fat-extraction flask by the small bulb and carefully decant as much as possible of the supernatant layer into the prepared fat-collecting vessel (see 9.3) containing a few boiling aids (6.10) in the case of a boiling or conical flask (optional with metal dishes). Avoid decanting any of the aqueous layer (see Figure 2).



**Key**

- 1 solvent
- 2 interface
- 3 aqueous layer
- a At second and third extraction.
- b At first extraction.

**Figure 1 — Before decanting**



**Key**

- 1 interface
- 2 aqueous layer
- a At second and third extraction.
- b At first extraction.

**Figure 2 — After decanting**

**9.4.10** Rinse the outside of the neck of the fat-extraction flask with a little mixed solvent (5.6). Collect the rinsings in the fat-collecting vessel. Take care that the mixed solvent does not spread over the outside of the fat-extraction flask. If desired, remove the solvent or a part of it from the fat-collecting vessel by distillation or evaporation as specified in 9.4.14.

**9.4.11** Add 5 ml of ethanol (5.2) to the contents of the fat-extraction flask. Using the ethanol, rinse the inside of the neck of the flask and mix as specified in 9.4.4.

**9.4.12** Carry out a second extraction by repeating the operations specified in 9.4.5 to 9.4.9 inclusive. Instead of 25 ml, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using the diethyl ether, also rinse the inner wall of the neck of the fat-extraction flask.

If necessary, raise the interface slightly to the middle of the stem of the flask by gently adding water down the side of the flask (see Figure 1) to enable the final decanting of solvent to be as complete as possible (see Figure 2).

**9.4.13** Carry out a third extraction without addition of ethanol by again repeating the operations specified in 9.4.5 to 9.4.9 inclusive. Again, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using the diethyl ether, rinse the inside of the neck of the fat-extraction flask again.

If necessary, raise the interface slightly to the middle of the stem of the flask by gently adding water down the side of the flask (see Figure 1) to enable the final decanting of solvent to be as complete as possible (see Figure 2).

NOTE The third extraction may be omitted for products with a fat content of less than 5 % mass fraction.

**9.4.14** Remove the solvents (including the ethanol) as completely as possible from the fat-collecting vessel, by distillation if using a boiling or conical flask, or by evaporation if using a beaker or dish (6.3). Rinse the inside of the neck of the boiling or conical flask with a little mixed solvent (5.6) before commencing the distillation.

**9.4.15** Heat the fat-collecting vessel, with the boiling or conical flask placed on its side to allow solvent vapour to escape, for 1 h in the drying oven (6.4) maintained at 102 °C. Remove the fat-collecting vessel from the oven and immediately verify whether or not the fat is clear. If the fat is not clear, fatty extraneous matter is presumed to be present and the whole procedure shall be repeated. If the fat is clear, protect the fat-collecting vessel from dust and allow the fat-collecting vessel to cool (preferably not in a desiccator) to the temperature of the weighing room (a glass fat-collecting vessel for at least 1 h, a metal dish for at least 30 min).

Do not wipe the fat-collecting vessel immediately before weighing. Use tongs (6.13) to place the fat-collecting vessel on the balance. Weigh the fat-collecting vessel to the nearest 1,0 mg.

**9.4.16** Heat the fat-collecting vessel, with the boiling or conical flask placed on its side to allow solvent vapour to escape, for a further 30 min in the drying oven (6.4) maintained at 102 °C. Cool and reweigh as specified in 9.4.15. If necessary, repeat the heating and weighing procedures until the mass of the fat-collecting vessel decreases by 1,0 mg or less, or increases between two successive weighings. Record the minimum mass as the mass of the fat-collecting vessel and extracted matter.

## 10 Calculation and expression of results

### 10.1 Calculation

Calculate the fat content,  $w_f$ , expressed as a percentage mass fraction, of the sample using Equation (1):

$$w_f = \frac{(m_1 - m_2) - (m_3 - m_4)}{m_0} \times 100 \quad (1)$$

where

$m_0$  is the mass, in grams, of the test portion (9.1);

$m_1$  is the mass, in grams, of the fat-collecting vessel and extracted matter, determined in 9.4.16;

$m_2$  is the mass, in grams, of the prepared fat-collecting vessel (9.3);

$m_3$  is the mass, in grams, of the fat-collecting vessel used in the blank test (9.2) and any extracted matter determined in 9.4.16;

$m_4$  is the mass, in grams, of the fat-collecting vessel (9.3) used in the blank test (9.2).

## 10.2 Expression of results

Round the result to two decimal places.

## 11 Precision

### 11.1 Interlaboratory test

Details of an interlaboratory test in accordance with ISO 5725:1986<sup>1)</sup> (see Reference [2]) on the precision of the method appear in Reference [4].

The values for repeatability and reproducibility limits are expressed for the 95 % probability level and may not be applicable to concentration ranges and matrices other than those given.

### 11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will not in more than 5 % of cases be greater than a mass fraction of fat of:

- a) 0,20 % for dried high-fat milk and dried whole milk;
- b) 0,15 % for dried partially skimmed milk and dried buttermilk;
- c) 0,10 % for dried skimmed milk and dried whey.

### 11.3 Reproducibility

The absolute difference between two independent single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will not in more than 5 % of cases be greater than a mass fraction of fat of:

- a) 0,30 % for dried high-fat milk and dried whole milk;
- b) 0,25 % for dried partially skimmed milk and dried buttermilk;
- c) 0,20 % for dried skimmed milk and dried whey.

## 12 Test report

The test report shall include at least the following information:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, together with reference to this International Standard;
- d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- e) the corrections made, if a value of more than 2,5 mg is obtained in the blank test for the method;
- f) the test result(s) obtained or, if the repeatability has been checked, the final quoted result obtained.

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1) ISO 5725:1986 (now superseded) was used to obtain the precision data.

## **Annex A** (informative)

### **Notes on procedures**

#### **A.1 Blank test to check the reagents (see 9.2.2)**

In this blank test, a fat-collecting vessel for mass control purposes has to be used so that changes in the atmospheric condition of the balance room or temperature effects of the fat-collecting vessel do not falsely suggest the presence or absence of non-volatile matter in the extract of the reagents. This fat-collecting vessel may be used as a counterweight vessel in the case of a two-pan balance. Otherwise, deviations of the apparent mass ( $m_3 - m_4$  in 10.1) of the fat-collecting vessel for control purposes should be considered when checking the mass of the fat-collecting vessel used for the blank test. Hence, the change in apparent mass of the fat-collecting vessel, corrected for the apparent change in mass of the fat-collecting vessel for control purposes, shall show no increase in mass greater than 1,0 mg.

Very occasionally, the solvents may contain volatile matter which is strongly retained in fat. If there are indications of the presence of such substances, carry out blank tests on all the reagents and for each solvent using a fat-collecting vessel with about 1 g of anhydrous butterfat. If necessary, redistil solvents in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Use the solvents only shortly after redistillation.

#### **A.2 Blank test carried out simultaneously with the determination (see 9.2.1)**

The value obtained in the blank test, carried out simultaneously with the determination, enables the apparent mass of substances extracted from a test portion ( $m_1 - m_2$ ) to be corrected for the presence of any non-volatile matter derived from the reagents and also for any change of atmospheric conditions in the balance room and some temperature difference between the fat-collecting vessel and the balance room at the two weighings (9.4.16 and 9.3).

Under favourable conditions (low value in the blank test on reagents, constant temperature of the balance room, sufficient cooling time for fat-collecting vessel), the value will usually be less than 1,0 mg and can then be neglected in the calculation in the case of routine determinations. Slightly higher values (positive and negative) up to 2,5 mg are also often encountered. After correction for these values, the results will still be accurate. Corrections of more than 2,5 mg should be mentioned in the test report (Clause 12).

If the value obtained in this blank test regularly exceeds 1,0 mg, the reagents should be checked, if no recent check has been made. Any impure reagent or reagents traced should be replaced or purified (see 9.2.2 and Clause A.1).

#### **A.3 Test for peroxides**

To test for peroxides, add 1 ml of a freshly prepared 100 g/l potassium iodide solution to 10 ml of diethyl ether (5.4) in a small glass-stoppered cylinder which has been previously rinsed with the ether. Shake the cylinder and allow to stand for 1 min. No yellow colour should be observed in the diethyl ether layer.

Other suitable methods of testing for peroxides may be used.

To ensure that the diethyl ether is free, and is maintained free, from peroxides, treat the diethyl ether, at least 3 days before it is to be used, as follows.

Cut zinc foil into strips that will reach at least halfway up the bottle containing the diethyl ether, using approximately 8 000 mm<sup>2</sup> of foil per litre of diethyl ether.

Before use, completely immerse the strips of foil for 1 min in a solution containing 10 g of copper(II) sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and 2 ml of concentrated (98 % mass fraction) sulfuric acid per litre. Wash the strips gently but thoroughly with water, place the wet copper-plated strips in the bottle containing the diethyl ether, and leave the strips in the bottle.

Other methods may be used provided that they do not affect the result of the determination.

#### **A.4 Diethyl ether containing antioxidants**

Diethyl ether containing about 1 mg of antioxidants per kilogram is available in some countries, especially for fat determinations. This content does not exclude its use for reference purposes.

In other countries, diethyl ether with higher antioxidant contents, e.g. up to 7 mg/kg, is available. Such ether should only be used for routine determinations with an obligatory blank test carried out simultaneously with the determination(s) to correct for systematic errors due to the antioxidant residue. For reference purposes, such diethyl ether shall always be distilled before use.

#### **A.5 Ethanol**

Ethanol denatured otherwise than by the addition of methanol may be used, provided that the denaturant does not affect the result of the determination.

## **Annex B** (informative)

### **Alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings**

#### **B.1 General**

If fat-extraction tubes with siphon or wash-bottle fittings are to be used, use the procedure specified in this annex. The tubes shall be provided with good quality cork bungs or stoppers as specified for the flasks in 6.6 (see Figure B.1 as an example).

#### **B.2 Procedure**

##### **B.2.1 Preparation of test sample**

See Clause 8.

##### **B.2.2 Test portion**

Proceed as specified in 9.1, but using the fat-extraction tubes (see Note to 6.6 and Figure B.1).

The test portion shall be delivered as completely as possible to the bottom of the fat-extraction tube.

##### **B.2.3 Blank test**

See 9.2 and Clause A.2.

##### **B.2.4 Preparation of fat-collecting vessel**

See 9.3.

##### **B.2.5 Determination**

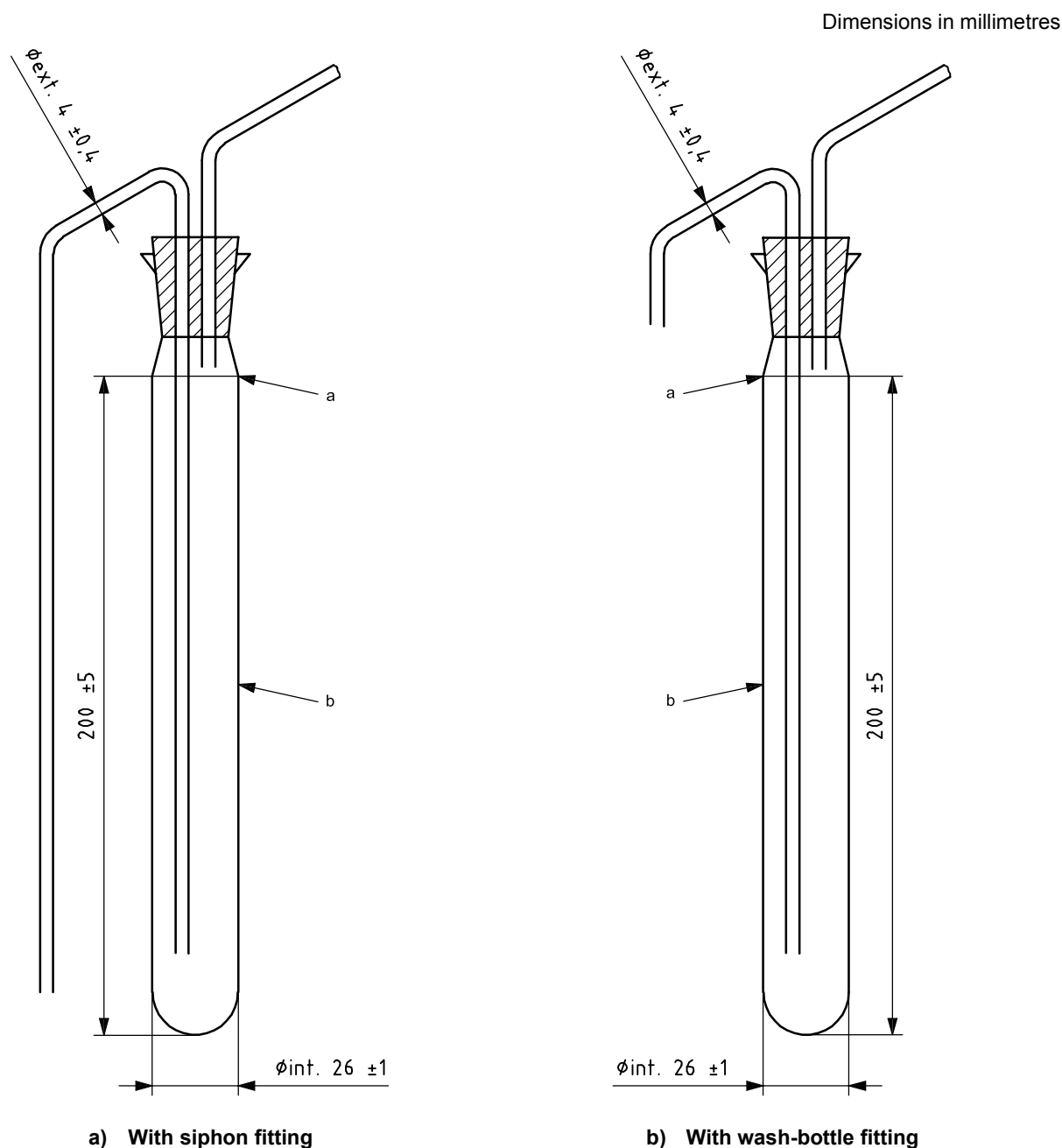
**B.2.5.1** Carry out the determination without delay.

Add 10 ml of water at  $65\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$  to the test portion in the fat-extraction tube (B.2.2) so as to wash the test portion on to the bottom of the tube. Mix thoroughly.

**B.2.5.2** Add 2 ml of ammonia solution (5.1) to the test portion in the fat-extraction tube (B.2.5.1), or an equivalent volume of a more concentrated ammonia solution (see Note to 5.1). Mix thoroughly with the pretreated test portion at the bottom of the fat-extraction tube.

**B.2.5.3** Heat the tube at  $65\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$  in the water bath (6.5) for 15 min to 20 min with occasional shaking. Cool in running water to room temperature.

**B.2.5.4** Add 10 ml of ethanol (5.2). Mix gently but thoroughly with the mixture at the bottom of the fat-extraction tube. If desired, add 2 drops of the Congo red solution (5.3).



a Capacity to this level with fittings removed 105 ml  $\pm$  5 ml.

b Wall thickness 1,5 mm  $\pm$  0,5 mm.

**Figure B.1 — Examples of fat-extraction tubes**

**B.2.5.5** Add 25 ml of diethyl ether (5.4). Close the fat-extraction tube with a cork bung saturated with water or with a stopper of other material wetted with water (6.6). Shake the tube vigorously, but not excessively, with repeated inversions, for 1 min, to avoid the formation of persistent emulsions. If necessary, cool the tube in running water. Carefully remove the bung or stopper and rinse it and the neck of the tube with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the tube.

**B.2.5.6** Add 25 ml of the light petroleum (5.5). Close the fat-extraction tube with the rewetted (by dipping in water) bung or stopper. Shake the tube gently for 30 s, as described in B.2.5.5.

**B.2.5.7** Centrifuge the closed fat-extraction tube for 1 min to 5 min at a radial acceleration of 80*g* to 90*g*. If a centrifuge (6.2) is not available, allow the closed tube to stand in the rack (6.7) for at least 30 min until the supernatant layer is clear and distinctly separated from the aqueous layer. If necessary, cool the tube in running water to room temperature.

**B.2.5.8** Carefully remove the bung or stopper and rinse it and the neck of the fat-extraction tube with a little mixed solvent (5.6). Use the wash bottle (6.8) so that the rinsings run into the tube.

**B.2.5.9** Insert a siphon fitting or a wash-bottle fitting into the fat-extraction tube. Push down the long inner limb of the fitting until the inlet is approximately 4 mm above the interface between the layers. The inner limb of the fitting shall be parallel to the axis of the fat-extraction tube.

Carefully transfer the supernatant layer out of the fat-extraction tube into the fat-collecting vessel (see 9.3) containing a few boiling aids (6.10) in the case of boiling or conical flasks (optional with metal dishes). Avoid the transfer of any of the aqueous layer. Rinse the outlet of the fitting with a little mixed solvent, collecting the rinsings in the fat-collecting vessel.

**NOTE** The supernatant layer can be transferred out of the fat-extraction tube by using, for example, a rubber bulb attached to the short stem to apply pressure.

**B.2.5.10** Loosen the fitting from the neck of the fat-extraction tube. Slightly raise the fitting and rinse the lower part of its long inner limb with a little mixed solvent (5.6). Lower and re-insert the fitting and transfer the rinsings to the fat-collecting vessel.

Rinse the outlet of the fitting with a little mixed solvent again, collecting the rinsings in the fat-collecting vessel. If desired, remove the solvent or a part of it from the fat-collecting vessel by distillation or evaporation as specified in 9.4.14.

**B.2.5.11** Again loosen the fitting from the neck. Slightly raise the fitting and add 5 ml of ethanol to the contents of the fat-extraction tube. Use the ethanol to rinse the long inner limb of the fitting. Mix as described in B.2.5.4.

**B.2.5.12** Carry out a second extraction by repeating the operations described in B.2.5.5 to B.2.5.10. Instead of 25 ml, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using the diethyl ether, rinse the long inner limb of the fitting during the removal of the fitting from the fat-extraction tube after the previous extraction.

**B.2.5.13** Carry out a third extraction without the addition of ethanol by again repeating the operations described in B.2.5.5 to B.2.5.10. Again, use only 15 ml of diethyl ether (5.4) and 15 ml of light petroleum (5.5). Using the diethyl ether, rinse the long inner limb of the fitting as described in B.2.5.12.

**NOTE** The third extraction can be omitted for milk with a fat content of less than 5 % mass fraction.

**B.2.5.14** Proceed as specified in 9.4.14 to 9.4.16.

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2) Superseded.



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